1083-8791/06/1201-0103$32.00/0
doi:10.1016/j.bbmt.2005.10.025

Adoptive Transfer of Allogeneic Antigen-Specific T Cells

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ABSTRACT

Animal models and human studies of allogeneic hematopoietic cell transplantation (HCT) demonstrate that immunologic nonidentity between donor and recipient is responsible for a graft versus leukemia (GVL) effect that contributes to complete tumor eradication. A variety of immune cells have been implicated in the GVL effect including NK cells, B cells, and $CD4^+$ and $CD8^+$ T cells that recognize minor histocompatibility (H) or leukemia-associated antigens. Here we discuss strategies for employing T cells specific for minor H antigens to augment the GVL effect.

KEY WORDS

Adoptive transfer • T-cell response

INTRODUCTION

The ability of allogeneic hematopoietic cell transplantation (HCT) to cure leukemia represents a conspicuous example of the capacity of the human immune system to destroy tumors. In allogeneic HCT between HLA-identical individuals, recognition by donor T cells of minor histocompatibility (H) antigens, which are peptides derived from endogenous proteins and presented by major histocompatibility complex (MHC) molecules on recipient cells, provides a mechanism by which graft-versus-leukemia (GVL) effects can be mediated [1]. Several features of minor H antigens suggest that they are attractive targets for antitumor therapy. First, minor H antigens are highly immunogenic, and donor T cells cause graft-versus-host disease (GVHD) and mediate GVL despite the administration of immunosuppressive drugs to block alloreactivity. Second, most minor H antigen-specific T-cell clones have high avidity for their cognate antigen, thus increasing the likelihood they will recognize tumor cells that may express low levels of MHC. Third, the T-cell response to minor H antigens involves both $CD8^+$ and $CD4^+$ T-cell subsets and is frequently directed at multiple determinants. The breadth of recognition may prevent the outgrowth of tumor cells that have lost or reduced levels of antigen expression, which has been observed in immunotherapy studies that target only a single determinant. However, T-cell recognition of minor H antigens is also responsible for GVHD [2], and a prevailing challenge in allogeneic HCT is to develop approaches that will permit separation of the GVL effect from GVHD or abrogate GVHD after tumor eradication is complete.

MOLECULAR CHARACTERIZATION OF HUMAN MINOR H ANTIGENS

An improved understanding of the molecular nature and tissue expression of minor H antigens may facilitate efforts to manipulate GVL activity and separate it from GVHD. A variety of methods, including complementary DNA expression cloning, peptide elution and mass spectrometry, and genetic linkage analysis, have been used for identifying the polymorphic genes that encode minor H antigens. Although fewer than 30 genes that encode minor H antigens have been discovered, these efforts are providing insight into the mechanisms by which genetic polymorphism results in immunogenicity, including differential gene expression, alterations in MHC binding or T cell–receptor contact, alterations in proteosomal processing or peptide transport, and peptide splicing and reassortment [3-7; unpublished data]. Translating this information into strategies that induce a GVL effect based on augmenting T-cell responses to minor H antigens has proven more difficult. Some minor H antigens exhibit

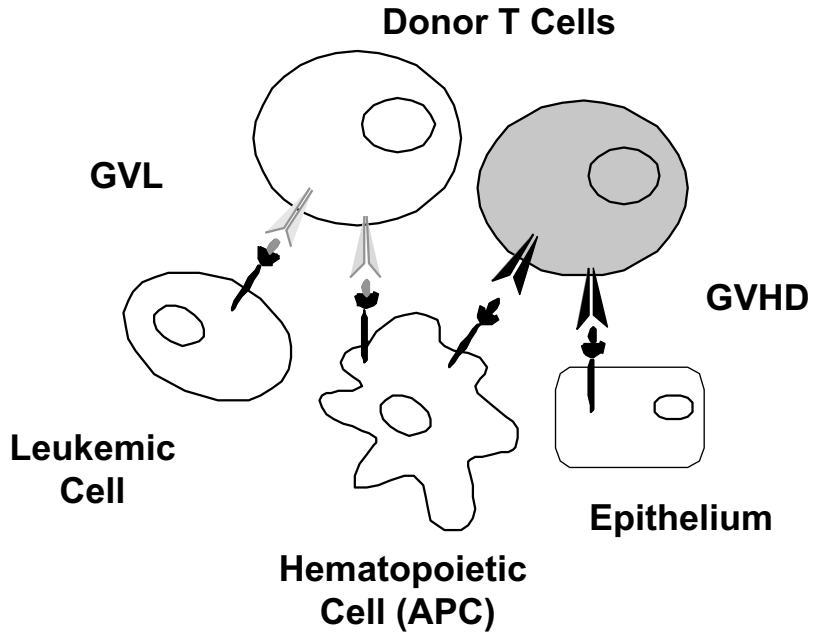

Figure 1. Separation of GVL effects and GVHD according to tissue expression of minor H antigens. T cells that recognize minor H antigens, which are selectively expressed on hematopoietic cells, including leukemic cells, will mediate a GVL effect without inducing damage to epithelial cells. By contrast, T cells that recognize broadly expressed minor H antigens may induce GVHD alone or concomitant with a GVL effect. APC, antigen-presenting cell.

preferential expression on hematopoietic cells, including leukemic stem cells [8], and a simple model is that the tissue expression of minor H antigens might provide the basis for segregating the GVL effect from GVHD [9]. T cells that recognize minor H antigens that are selectively expressed by recipient hematopoietic cells, including leukemic cells, could contribute to the elimination of leukemia without GVHD. Alternatively, T cells that recognize antigens that are broadly expressed by both hematopoietic cells and epithelium might contribute to both GVL and GVHD, or GVHD alone (Figure 1).

TUMOR-REACTIVE T-CELL RESPONSES AFTER NONMYELOABLATIVE HCT

Transplantation of donor peripheral blood stem cells after nonmyeloablative conditioning (NM-HCT) represents a circumstance in which donor immune cells assume paramount importance for tumor eradication [10], and it provides a distinct opportunity to characterize the responses that recognize tumor cells. NM-HCT is particularly effective for indolent hematologic malignancies such as chronic lymphocytic leukemia (CLL), in which tumor cells are also readily accessible for analysis. In patients receiving NM-HCT for CLL, we have analyzed the kinetics, phenotype, and specificity of alloreactive and tumor-reactive T-cell responses. A rapid decline in circulating CLL cells is observed in most patients after transplantation, including those with advanced disease and a high tumor burden. The antitumor effects of transplantation coincide with the development of donor T-cell chimerism and are often temporally dissociated from GVHD. In responding patients, $CD4^+$ and $CD8^+$ T cells reactive with recipient CLL cells are detected in the blood early after transplantation and persist for >1 year. Individual T-cell clones isolated from responding patients exhibit cytolytic activity against recipient CLL cells, and most clones recognize minor H antigens, whereas a smaller subset seem to recognize tumor-specific determinants. These studies demonstrate that many patients develop potent, early, and sustained alloreactive and tumor-reactive T-cell responses after NM-HCT that correlate with tumor clearance. Unfortunately, not all patients respond to NM-HCT, and it is anticipated the isolation and characterization of T cells in responding patients will enable the development of targeted approaches based on adoptive T-cell transfer or vaccination to enhance tumor-reactive T cells after transplantation in nonresponding patients.

ADOPTIVE TRANSFER OF ALLOREACTIVE T-CELL CLONES

Myeloablative regimens are preferred for patients with acute leukemia undergoing allogeneic HCT, but relapse-free survival still relies in part on a GVL effect [11,12]. For patients with advanced acute leukemia at the time of HCT, relapse remains a major cause of failure, and strategies to augment the GVL effect are needed. On the basis of previous work demonstrating

that the adoptive transfer of donor-derived cytomegalovirus-specific cytotoxic T lymphocyte (CTL) clones can restore functional cytomegalovirus-specific immunity [13], we have investigated the adoptive transfer of T-cell clones specific for minor H antigens to treat patients with relapse of acute leukemia after allogeneic HCT. At the present time, too few hematopoietic lineage-restricted minor H antigens have been molecularly characterized to prospectively evaluate them as targets for therapy [14,15]. However, $CD8^+$ CTL clones recognizing recipient minor H antigens can be isolated after transplantation from most patients undergoing allogeneic HCT from an MHC-matched, related donor [16], and a subset recognize recipient hematopoietic cells but not nonhematopoietic cells in vitro, thus suggesting that they may be specific for hematopoietic lineage-specific genes. It is not possible to rapidly identify the genes encoding the antigens and comprehensively analyze tissue expression in all patients who relapse. Thus, the safety of adoptively transferring CTLs selected on the basis of recognition of recipient hematopoietic target cells but not skin fibroblasts to patients with acute leukemia who relapse after HCT is currently being investigated.

The most frequent toxicities observed with the infusion of minor H antigen–specific CTL clones have been fever and chills, which occurred in all patients, and pulmonary infiltrates, which occurred in a minority of patients. GVHD was considered the most likely toxicity of infusing minor H antigen–specific CTLs but occurred in a minority of patients on this study, and the role of T-cell infusions as a causative factor could not be definitively established in any of the patients. Thus, the strategy of selecting T-cell clones solely on the basis of in vitro assays will not be sufficient for ensuring safety in all patients. This illustrates the importance of ongoing efforts to molecularly characterize minor H antigens to identify additional antigens that are selectively expressed in hematopoietic cells as targets for immunotherapy.

A subset of patients treated with infusions of minor H antigen–specific CTLs had persistent leukemia after receiving chemotherapy for relapse and achieved a remission only after the T-cell infusions. Although these results provide encouraging evidence that transferred T-cell clones exert antileukemic activity, the patients later relapsed. The persistence of transferred T cells was measured by quantitative polymerase chain reaction that used primers to amplify clone-specific sequences of the T-cell receptor β gene. High levels of transferred cells were detected in the blood and bone marrow shortly after the cell infusions, but persistence of transferred T cells was not optimal. A short duration of in vivo persistence of transferred T-cell clones has been observed in other studies [17] and may contribute to the lack of sustained treatment efficacy.

A critical question with implications for efforts to use adoptive transfer of T-cell clones for cancer is whether the failure of minor H antigen–specific T cells to persist in vivo is due to intrinsic properties of differentiated effector T-cell clones that are acquired during prolonged ex vivo culture or due to extrinsic factors such as activation-induced cell death, inadequate CD4 T helper responses, or lack of prosurvival cytokines [18]. To address these issues and identify improved regimens for cell transfer that might be applied in humans, studies have been initiated in nonhuman primates in which antigen-specific T-cell clones are isolated and propagated under culture conditions identical to those of human T-cell clones. The clones are genetically marked before transfer to facilitate the analysis of their persistence and migration in vivo. Preliminary results of these studies demonstrate that differentiated effector T cells do retain the capacity to survive long-term in vivo. Moreover, the transferred T cells migrate widely to lymphoid organs and acquire phenotypic and functional characteristics of memory T cells. However, without exogenous cytokines, only a minor proportion of transferred cells are capable of persisting as memory cells when transferred into a full lymphoid compartment. Ongoing studies are addressing whether cytokines such as interleukin 2 or interleukin 15 or modifications of the host lymphoid environment can promote in vivo expansion and persistence of transferred T-cell clones [19,20]. It is anticipated these studies will instruct future efforts toward adoptive transfer of T-cell clones targeting leukemic cells after allogeneic HCT.

REFERENCES

1. Bleakley M, Riddell SR. Molecules and mechanisms of the graft-versus-leukaemia effect. *Nat Rev Cancer*. 2004;4:371-380.
2. Dickinson AM, Charron D. Non-HLA immunogenetics in hematopoietic stem cell transplantation. *Curr Opin Immunol*. 2005;17:517-525.
3. Murata M, Warren EH, Riddell SR. A human minor histocompatibility antigen resulting from differential expression due to a gene deletion. *J Exp Med*. 2003;197:1279-1289.
4. den Haan JM, et al. The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science*. 1998;279:1054-1057.
5. Wang W, et al. Human H-Y: a male-specific histocompatibility antigen derived from the SMCY protein. *Science*. 1995;269: 1588-1590.
6. Spierings E, et al. The minor histocompatibility antigen HA-3 arises from differential proteasome-mediated cleavage of the lymphoid blast crisis (Lbc) oncoprotein. *Blood*. 2003; 102:621-629.
7. Brickner AG, et al. The immunogenicity of a new human minor

histocompatibility antigen results from differential antigen processing. *J Exp Med*. 2001;193:195-206.
8. Bonnet D, et al. CD8(+) minor histocompatibility antigen-specific cytotoxic T lymphocyte clones eliminate human acute myeloid leukemia stem cells. *Proc Natl Acad Sci U S A*. 1999; 96:8639-8644.
9. Dickinson AM, et al. In situ dissection of the graft-versus-host activities of cytotoxic T cells specific for minor histocompatibility antigens. *Nat Med*. 2002;8:410-414.
10. Baron F, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol*. 2005;23:1993-2003.
11. Horowitz MM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*. 1990;75:555-562.
12. Randolph SS, et al. Female donors contribute to a selective graft-versus-leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplants. *Blood*. 2004;103:347-352.
13. Walter EA, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med*. 1995; 333:1038-1044.
14. Marijt WA, et al. Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc Natl Acad Sci U S A*. 2003;100:2742-2747.
15. Akatsuka Y, et al. Identification of a polymorphic gene, BCL2A1, encoding two novel hematopoietic lineage-specific minor histocompatibility antigens. *J Exp Med*. 2003;197:1489-1500.
16. Warren EH, Greenberg PD, Riddell SR. Cytotoxic T-lymphocyte-defined human minor histocompatibility antigens with a restricted tissue distribution. *Blood*. 1998;91:2197-2207.
17. Dudley ME, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science*. 2002;298:850-854.
18. Ho WY, et al. Adoptive immunotherapy: engineering T cell responses as biologic weapons for tumor mass destruction. *Cancer Cell*. 2003;3:431-437.
19. Dudley ME, Rosenberg SA. Adoptive-cell-transfer therapy for the treatment of patients with cancer. *Nat Rev Cancer*. 2003;3: 666-675.
20. Klebanoff CA, et al. IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8+ T cells. *Proc Natl Acad Sci U S A*. 2004;101:1969-1974.